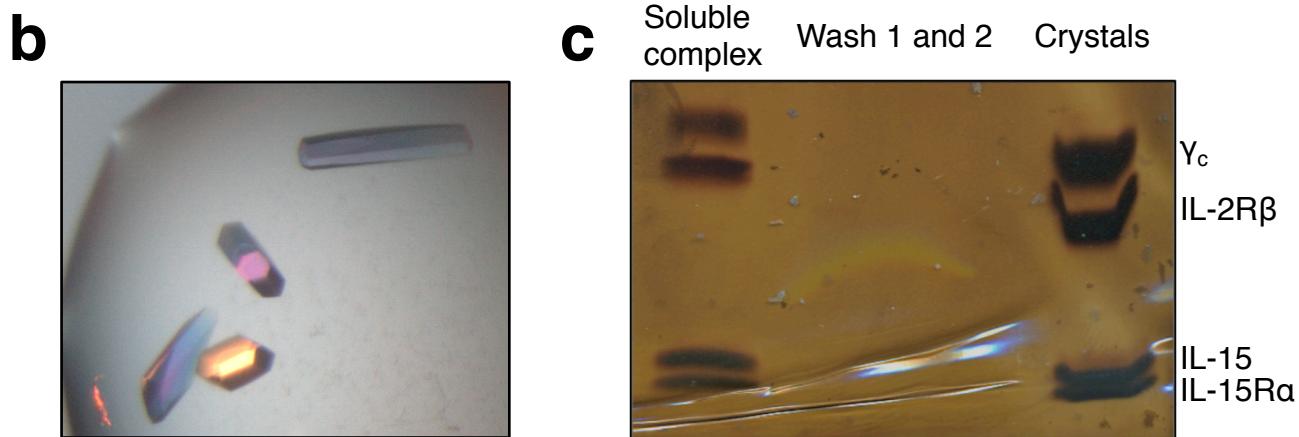
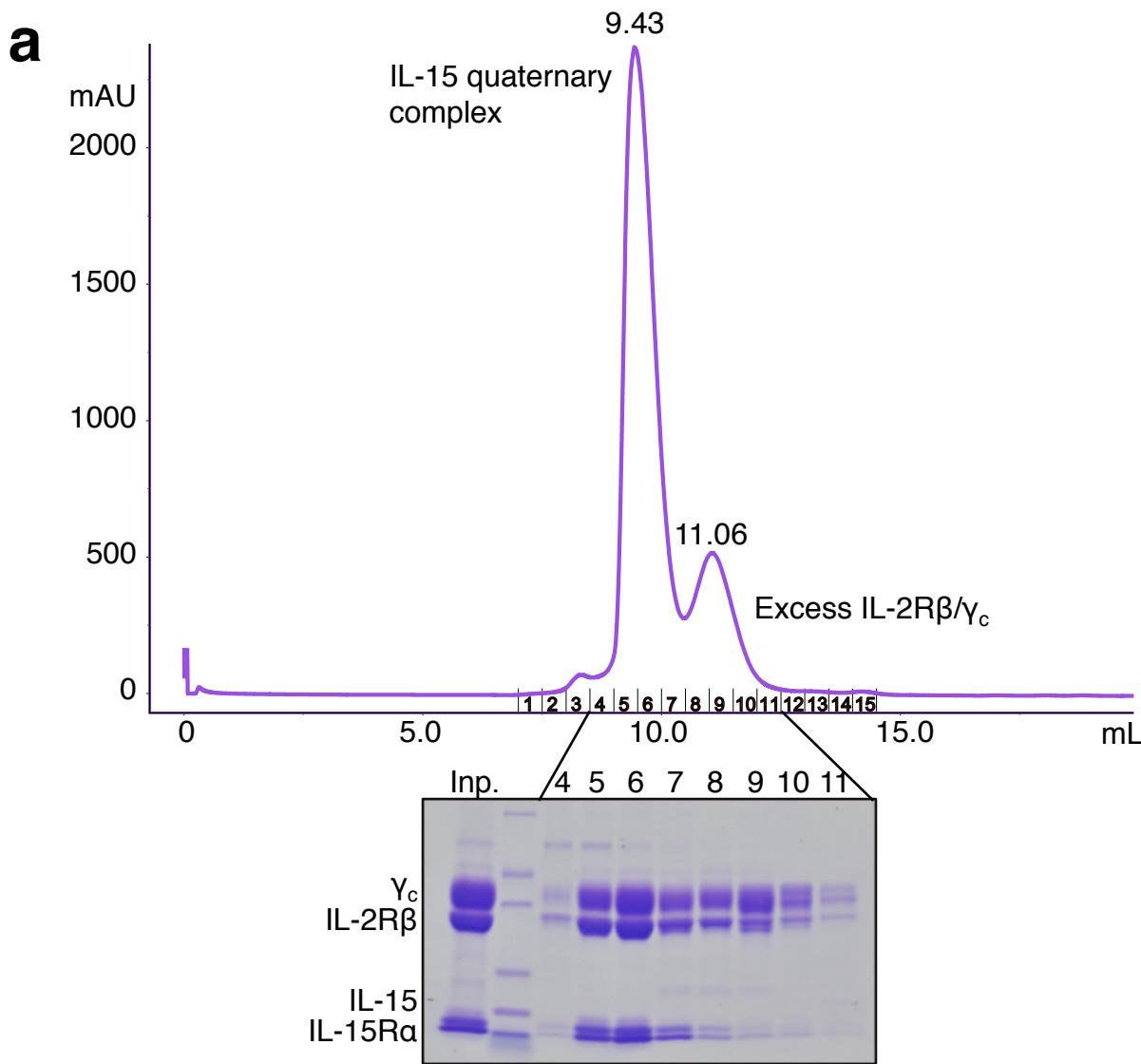


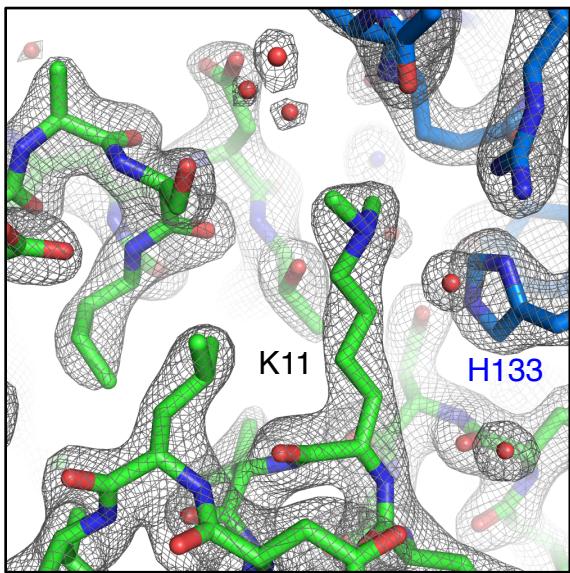
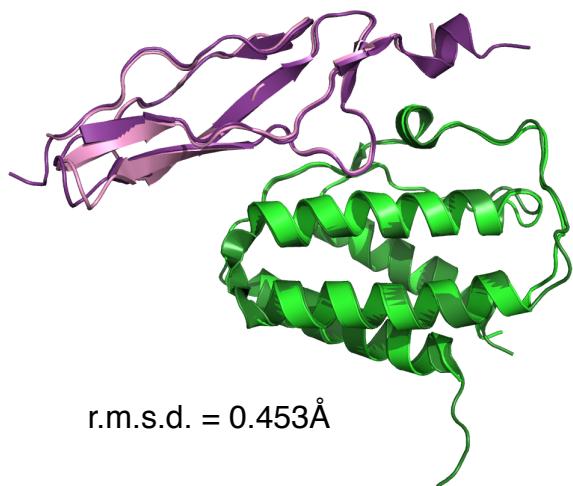
## **Supplementary Information**

### **Mechanistic and structural insight into the functional dichotomy between interleukin-2 and interleukin-15**

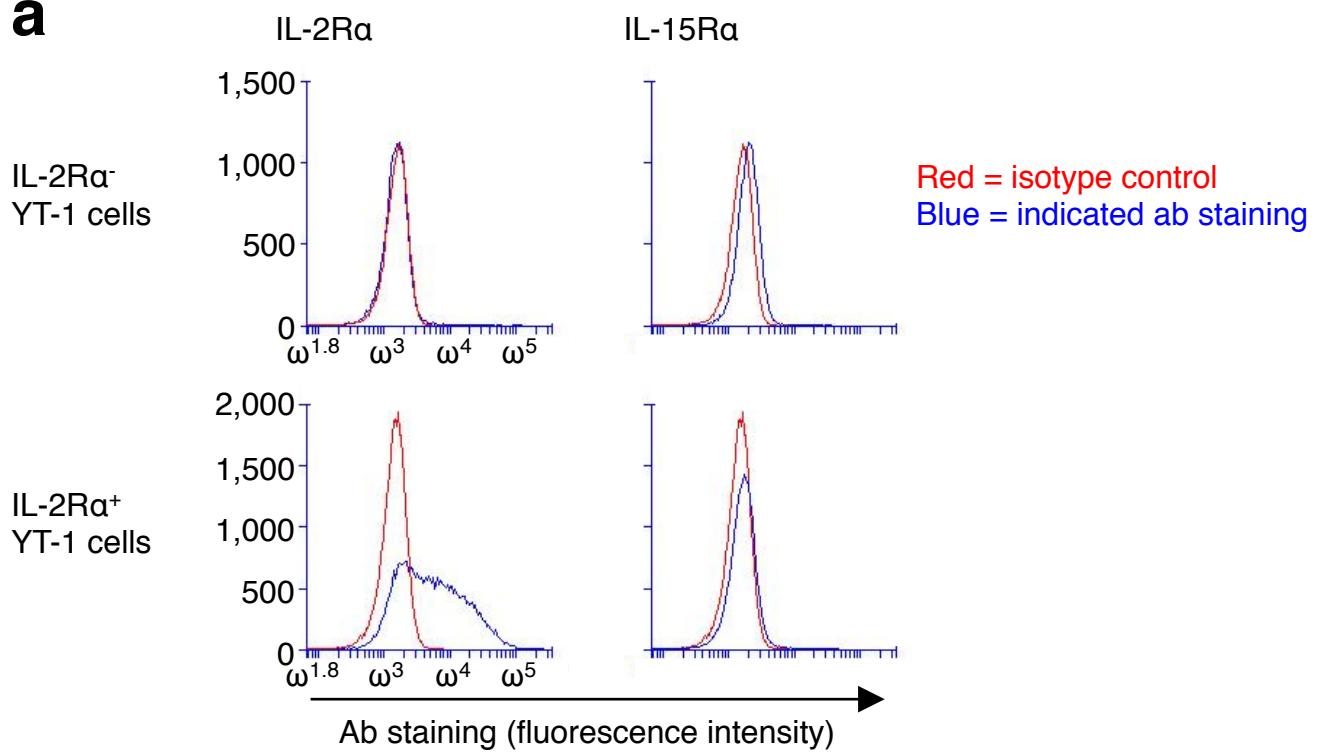
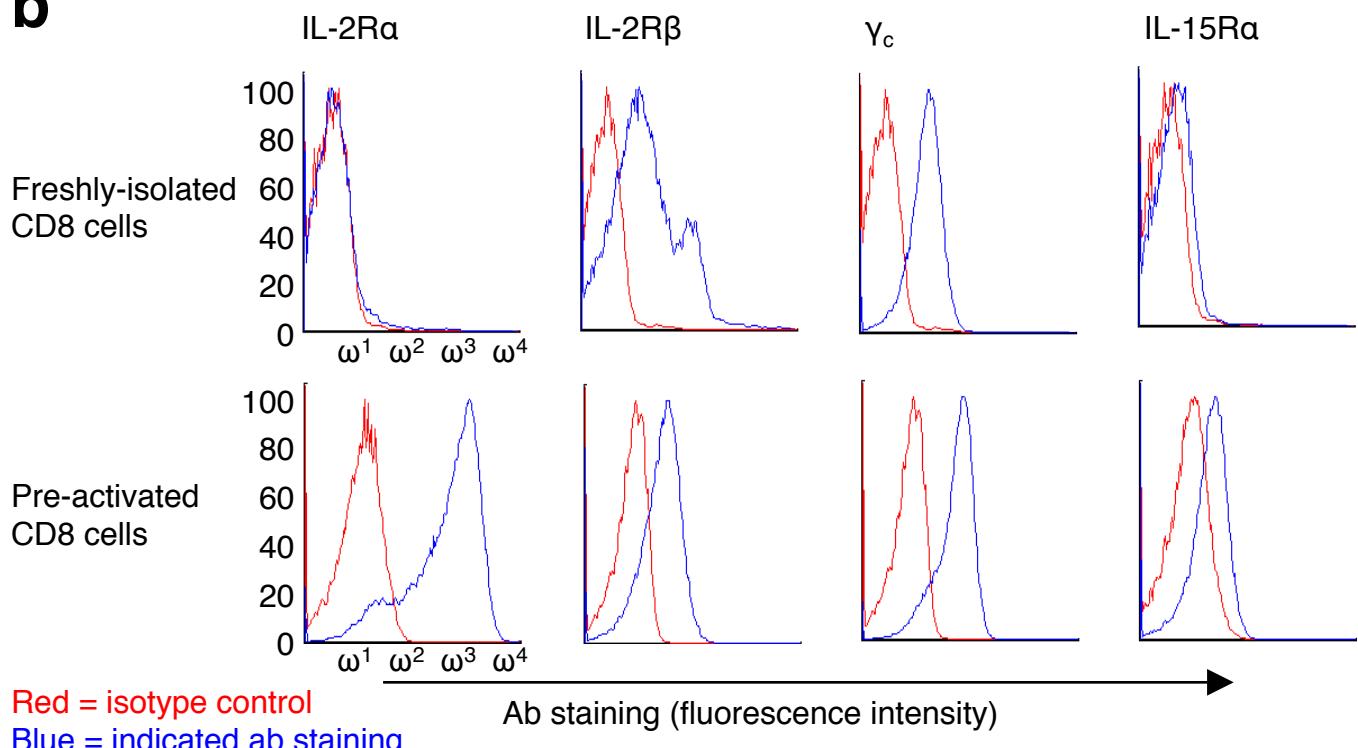
Aaron M. Ring, Jian-Xin Lin, Dan Feng, Suman Mitra, Mathias Rickert, Gregory R. Bowman, Vijay S. Pande, Peng Li, Ignacio Moraga<sup>1</sup>, Rosanne Spolski, Engin Özkan, Warren J. Leonard & K. Christopher Garcia



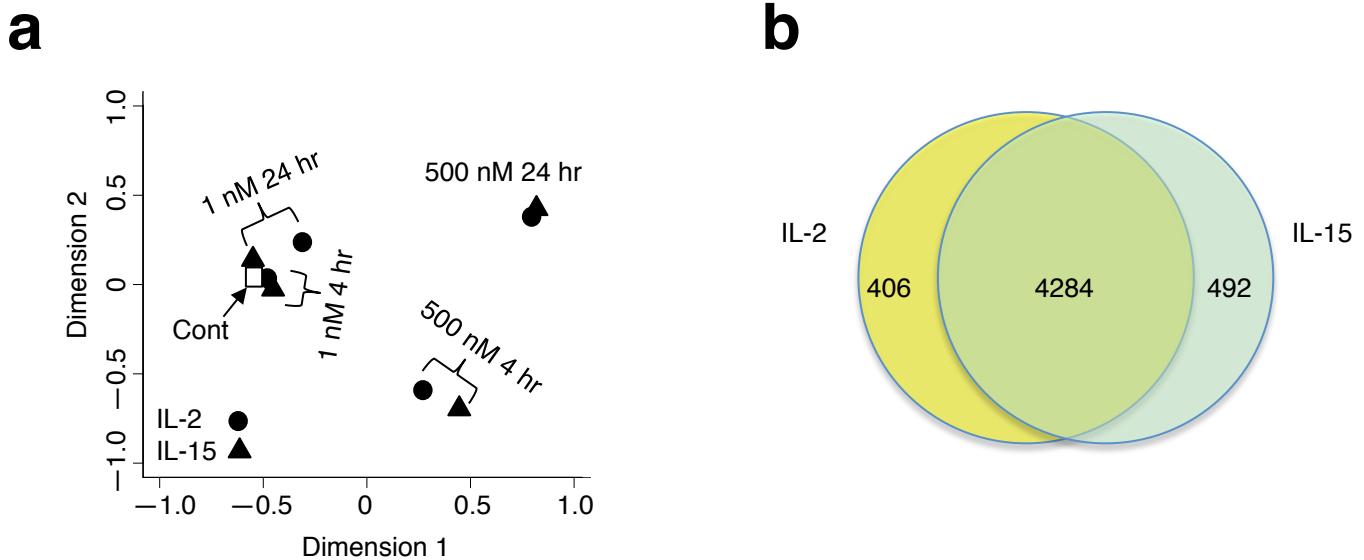
**Supplementary Figure 1.** Biochemical characterization of methylated IL-15 complex. (a) Size exclusion chromatograph (Superdex S75 column) of methylated IL-15 quaternary complex and Coomassie-stained SDS-PAGE gel of fractions indicates that lysine methylation does not abrogate complex formation. (b) Birefringent crystals of methylated IL-15 quaternary complex viewed under a polarizing lens. (c) Silver-stained gel of methylated IL-15 quaternary complex crystals indicates all components of the complex are present in the crystals.

**a**2mF<sub>o</sub>-DF<sub>c</sub> electron density map at 1.5 $\sigma$ :**b**

**Supplementary Figure 2.** Electron density of IL-15 quaternary complex and structural alignment with IL-15 binary complex. (a) 2mFo-DFc electron density map contoured at 1.5 $\sigma$ . (b) Superposition of binary IL-15:IL-15R $\alpha$  complex (dark green and light pink; PDB 2Z3Q) and IL-15:IL-15R $\alpha$  from the quaternary IL-15 complex (green and magenta).

**a****b**

**Supplementary Figure 3.** Receptor expression on YT-1 cells and mouse CD8 cells. (a) Flow cytometry histograms indicating expression of IL-2Ra (left column) and IL-15Ra (right column) in IL-2Ra $^-$  (top row) and IL-2Ra $^+$  (bottom row) YT-1 cells. X-axes represent fluorescence staining (log scale); Y-axes represent absolute number of cells. (b) Flow cytometry histograms indicating expression of IL-2Ra (left column), IL-2R $\beta$  (left middle column),  $\gamma_c$  (right middle column), and IL-15Ra (right column) on freshly-isolated (top row) or anti-CD3 ‘pre-activated’ (bottom row) mouse CD8 T cells. X-axes represent fluorescence intensity (log scale); Y-axes represent number of cells as a percentage of maximum.



**Supplementary Figure 4.** RNA-Seq summary of IL-2 and IL-15 gene expression profiles. (a) Two-dimensional Multidimensional scaling (MDS) plot of IL-2 and IL-15 regulated genes. (b) Venn diagram shows most of genes (4284) are regulated by both IL-2 and IL-15; however, some genes are more potently regulated by IL-2 (406) or IL-15 (492).

<b>Structural element</b>	<b>Residues</b>
Helix A	1-19
A-B Loop	20-36
Helix B	37-56
Helix C	57-77
C-D Loop	78-96
Helix D	97-112

**Supplementary Table 1.** Residue assignments for molecular dynamics simulations. The residue ranges (right column) comprising each structural element (left column) for molecular dynamics r.m.s.d. calculations are indicated.

**RNA-Seq cDNA synthesis:**

Primer Name	Sequence (5' to 3')
UP1	ATATGGATCCGGCGCGCCGTCGACTTTTTTTTTTTTTTTTTTT
UP2	ATATCTCGAGGGCGCGCCGGATCCTTTTTTTTTTTTTTT
AUP1*	(NH <sub>2</sub> )ATATGGATCCGGCGCGCCGTCGACTTTTTTTTTTTTTTT
AUP2*	(NH <sub>2</sub> )ATATCTCGAGGGCGCGCCGGATCCTTTTTTTTTTTTT

**qPCR validation:**

Primer Name	Sequence (5' to 3')
<i>Cish</i> FW (522)	GATCTGCTGTGCATGCCAAGA
<i>Cish</i> RV (602)	GGCCTCGCTGGCTGTAATAG
<i>Cish</i> TP (546)	(6-FAM)TTCTCCTACCTCGGAAATCTGG(Tamra-Q)
<i>Socs2</i> FW (844)	CCAGAACCCCCACGGAAT
<i>Socs2</i> RV (944)	TTGTTAATGGCGAGTCGACAGA
<i>Socs2</i> TP (865)	(6-FAM)ACTGTTCACCTGTACCTGACCAAACC(Tamra-Q)
<i>Gbp2</i> FW (2120)	CTGTGTGCAGAACATGCATGTATGA
<i>Gbp2</i> RV (2200)	TATGGCTGGGCATGATGAAG
<i>Gbp2</i> TP (2153)	(6-FAM)AGTCACCTGTGTTAGGTACTCTGG(Tamra-Q)
<i>Ilgp1</i> v1 FW (1639)	CCGTGTTCAAGGTTAGCATG
<i>Ilgp1</i> v1 RV (1719)	CCCCCAGACTCATTTCATG
<i>Ilgp1</i> v1 TP (1663)	(6-FAM)TGTTTAGTTGTCAACCCAACAGAC(Tamra-Q)
<i>Il2ra</i> FW (1114)	TAAACTGGACTTGCCATTGAAGA
<i>Il2ra</i> RV (1194)	GGGCCTCTCTCCCATTAAA
<i>Il2ra</i> TP (1144)	(6-FAM)TCTAACCACTTCAGCACAGCAGTTCTA(Tamra-Q)
<i>Tnf</i> FW (545)	CAGCCGATGGGTTGTACCTT
<i>Tnf</i> RV (625)	TGTGGGTGAGGAGCACGTAGT
<i>Tnf</i> TP (579)	(6-FAM)TCTCTCAAGGGACAAGGCTGCC(Tamra-Q)
<i>Ifng</i> FW (878)	CAAGCGGCTGACTGAAC
<i>Ifng</i> RV (958)	GGGTCACTGCAGCTGAATG
<i>Ifng</i> TP (900)	(6-FAM)TTGTAGCTTGACCTTACTTCAGTGA(Tamra-Q)
<i>Bcl2</i> FW (1949)	GGCATCTGCACACCTGGAT
<i>Bcl2</i> RV (2024)	ATCAAAGCCCAGACTCATTCAAC
<i>Bcl2</i> TP (1970)	(6-FAM)AGGATAACGGAGGCTGGGTAGGTGCAT(Tamra-Q)
<i>Gbp11</i> FW (2161)	TCACTGCTGGCCAATTAACTG
<i>Gbp11</i> RV (2241)	TGGGCTGAATGATCCTCACA
<i>Gbp11</i> TP (2185)	(6-FAM)AATCGAATAAGCTAGGAGACAAGGTTTC(Tamra-Q)

**Supplementary Table 2.** Primers sequences for RNA-Seq cDNA synthesis and qPCR validation.